



A German external quality survey of diagnostic microbiology of respiratory tract infections in patients with cystic fibrosis

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Abstract

Background: The goal of this pilot study was to design an external quality assessment (EQA) scheme for German cystic fibrosis (CF) clinical microbiology laboratories. Therefore, a multicentre study of 18 German CF laboratories was performed to evaluate their proficiency in analyzing CF respiratory secretions.

Methods: Simulated clinical specimens containing a set of four frequent CF pathogens, namely two *Pseudomonas aeruginosa* strains differing in morphotype (*mucoïd* versus *non-mucoïd*) and resistotype, one *Staphylococcus aureus* strain and one *Burkholderia multivorans* strain, were distributed to each laboratory. Isolation, identification and antimicrobial susceptibility testing (AST) of any bacterial pathogen present and completion of a questionnaire about applied microbiological protocols were requested.

Results: Three of four strains were isolated and identified correctly by almost all laboratories. *B. multivorans* was once misidentified as *Burkholderia cenocepacia*. Fourteen laboratories failed to detect the second multidrug resistant *P. aeruginosa* isolate. AST errors occurred most often for *P. aeruginosa* 2 followed by *B. multivorans*, *P. aeruginosa* 1 and *S. aureus*. Evaluation of the questionnaires revealed major differences in cultivation and identification techniques applied by the participating laboratories.

Conclusions: A periodical EQA programme for German CF laboratories and standardized microbiological procedures seem to be necessary to advance diagnostic microbiology employed on CF respiratory tract specimens and may help to improve anti-infective treatment and infection control practices for CF patients.

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1. Introduction

Cystic fibrosis (CF) is the most common hereditary disease in the Caucasian population and results from mutations in the CF transmembrane conductance regulator (CFTR) gene. The CFTR defect leads to an imbalance in the

epithelial electrolyte transport and multiple organ dysfunctions. In the CF lung, copious amounts of viscous respiratory secretions and impaired airway clearance cause recurrent bacterial infections with intense inflammation, airway obstruction, progressive lung tissue destruction and ultimately respiratory failure [1,2].

CF lung infection typically begins with the acquisition of *Staphylococcus aureus* and *Haemophilus influenzae*, followed by chronic *Pseudomonas aeruginosa* pneumonia, that remains the major cause of morbidity and mortality among CF patients [3,4]. In addition, *Burkholderia cepacia* complex, *Stenotrophomonas maltophilia*, *Achromobacter*

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xylosoxidans, non-tuberculosis mycobacteria and emerging pathogens like *Pandora* spp. and *Inquilinus limosus* further aggravate pulmonary infection in CF patients [5–9].

As a consequence of this progressive microbiological history periodical microbiological examinations of CF respiratory secretions remain cornerstones in the clinical management of CF lung disease [10–12].

Thus, accurate microbiological diagnostic employed on CF respiratory secretions is critical to ensure appropriate antimicrobial treatment and the implementation of reliable hygienic measures like segregation of patients with a respiratory tract culture that is positive for *P. aeruginosa* or *B. cepacia* complex.

During chronic stages of CF pneumonia microbiological examinations are complicated by the fact that respiratory samples obtained from CF patients often contain a mixture of different species and/or high quantities of diverse morphotypes and resistotypes, such as mucoid and non-mucoid *P. aeruginosa*, biochemical inactive, small colony, hypermutable and multidrug resistant (MDR) variants of pathogens that persisted for years [3,13,14]. Cultures performed on CF specimens commonly involve the use of many medium types, in particular selective media that facilitate the recovery of potential pathogens such as *S. aureus*, *P. aeruginosa* and *B. cepacia* complex from the complex microflora of CF airway secretions [15–19]. Therefore, the microbiological diagnosis of CF respiratory tract infections is labor-intensive and implies a wide experience in the field of CF microbiology.

About 8000 patients suffering from CF live in the Federal Republic of Germany and most of them are followed at about 120 different CF centres. In agreement, respiratory secretions of CF patients are analyzed in several microbiological laboratories all over Germany and on request by two consiliary laboratories on CF microbiology appointed by the Robert Koch Institute (in Munich: Consiliary Laboratory South, CLSth and in Hanover: Consiliary Laboratory North, CLNth).

To assess and continuously improve the quality of the microbiological laboratory performance in Germany, national external quality assessment (EQA) schemes are generally conducted for all kinds of routine microbiological procedures based on quality standards in microbiology-infectiology diagnostics, denoted as MiQ and issued by the German Society for Hygiene and Microbiology (DGHM). EQA trials are organized and performed by the INSTAND organization (Institute for Standardization and Documentation in Medical Laboratories) on behalf of the Board of the Federal Medical Council. However, during this study neither specific recommendations for clinical microbiology protocols, as available from American authorities, nor EQA schemes for CF respiratory tract samples existed in Germany [20,21].

In summary, the reliable microbiological diagnosis of CF lung infections imposes a particular challenge for clinical microbiology laboratories due to (i) the unique spectrum of typical CF pathogens and a variety of rarely occurring unusual non-fermentative bacteria, (ii) the broad phenotypic diversity of CF isolates descending from one initial infecting clone and (iii) the increasing selection of MDR variants. The

aim of this study was to design an EQA scheme for German microbiological laboratories regularly processing CF respiratory tract specimens. Therefore, a multilaboratory trial was organized by preparing simulated CF specimens that were sent by mail to 18 CF microbiological laboratories. The proficiency of participants was assessed regarding isolation, identification and antimicrobial susceptibility testing (AST) of classical CF pathogens. Moreover, they were asked to complete a questionnaire about applied “in-house” laboratory protocols.

In conclusion, the results of this study revealed important reporting errors in terms of CF test specimens and relevant differences in routine laboratory procedures and argue for the implementation of a continuous German EQA programme based on harmonized methodologies to improve the microbiological diagnosis of respiratory samples from CF patients.

2. Materials and methods

2.1. Bacterial strains and test specimens

Two identical freeze-dried simulated samples containing 4 bacterial strains of CF origin were distributed to each participating laboratory (for each strain cell densities of 1.0×10^8 /ml were intended). All strains were selected due to their definite biochemical differentiation profile by conventional and biochemical tests (API systems, BioMerieux). Each simulated specimen contained one strain of *S. aureus*, one strain of *Burkholderia multivorans* (API 20NE-code: 5067577/*B. cepacia* complex after 48 h) and two *P. aeruginosa* variants, one mucoid (termed *P. aeruginosa* 1, API 20NE-code: 1354575/*P. aeruginosa* after 48 h) and one non-mucoid of smaller colony size (termed *P. aeruginosa* 2, API 20NE-code: 1140575/*P. aeruginosa* after 48 h). *P. aeruginosa* 1 exhibited a mucoid, brown pigmented phenotype with susceptibility to all anti-pseudomonal antibiotics asked for, except ciprofloxacin, whereas *P. aeruginosa* 2 was selected for its non-mucoid, non-pigmented and MDR phenotype.

In August 2004, the test samples together with the questionnaires were sent within 12 h to 18 CF laboratories distributed all over Germany. The laboratories were instructed to add 500 µl trypticase soy broth, mix gently, incubate 15 min at 37 °C, direct samples to routine diagnostic procedures and report on potential pathogens within 3 weeks. Pre-treatment (e.g., with dithiothreitol) was not recommended. To test the stability of simulated samples during postal transport, specimens were stored at room temperature (RT) for 12 h and then at 4 °C and were investigated in triplicate at day 1 to day 7 by CLSth and CLNth, respectively. All four CF strains were reproducibly detected from each sample with individual colony forming units (CFU) of about $1.9 \times 10^8 \pm 2.8$ for *P. aeruginosa* 1, $2.9 \times 10^7 \pm 3.1$ for *P. aeruginosa* 2, $3.2 \times 10^8 \pm 1.2$ for *S. aureus* and $1.3 \times 10^8 \pm 1.2$ for *B. multivorans*. Antimicrobial susceptibility testing of the CF isolates was performed in triplicate by CLSth and CLNth. Minimal inhibitory concentrations (MICs) were determined

using agar dilution method according to CLSI (Clinical and laboratory standards institute) recommendations by CLSth [22] and microdilution method (Micronaut system, Merlin) by CLNth. MICs were interpreted as susceptible (S), intermediate (I) or resistant (R) according to CLSI breakpoints [23].

2.2. Report forms and questionnaires

A report form was provided for each of the four CF test strains included in the samples. Herein, 12 antimicrobial agents were listed and the participants were asked to report these agents and any other antibiotic agent they routinely test by using their standard AST procedures. Due to the estimated broad spectrum of AST methodologies, the microbiological laboratories were asked to score their AST results primarily as susceptible, intermediate and resistant but to append MIC values if available. The supplied questionnaire covered details of cultivation (e.g., media, cultivation temperature and time), species identification and AST procedures.

3. Results

Clinical microbiology laboratory results and protocols were provided by 17 of the 18 participating CF laboratories. Cultivation media, methods for species identification and AST of the laboratories and microbiological results of simulated CF specimens are summarized in Tables 1–4, respectively.

3.1. Laboratory results of bacterial cultivation and species identification

S. aureus and *P. aeruginosa* 1 were cultivated and correctly identified by all 17 reporting laboratories. *B. multivorans* was identified as a member of the *B. cepacia*

Table 1
Primary isolation media used by participants for cultivation of distributed specimens

Culture media	No. of laboratories
Liquid culture media	11
Solid media:	
Blood agar	17
Chocolate agar	17
Selective media for	
Gram-negative bacteria ^a	17
<i>B. cepacia</i> complex ^b	12
<i>P. aeruginosa</i> ^c	3
<i>S. aureus</i> ^d	8
Fungi ^e	17
Meropenem agar	1

^a MacConkey agar, cystine lactose-electrolyte-deficient agar, Winkle agar, Endoagar or not specified.

^b MAST selective agar, *Burkholderia cepacia* selective agar, or not specified.

^c Cetrimid or not specified.

^d Mannitol salt agar, Columbia colistin-nalidixic acid agar, Chapman agar or not specified.

^e Chromagar, Sabouraud agar, sulfite-polymyxin-sulfadiazine agar, erythritol-chloramphenicol agar, or not specified.

Table 2
Identification methods/systems used by participants

Identification system	No. of laboratories
<i>S. aureus</i>	
Conventional biochemical tests	9
Api ID32Staph (BioMerieux)	3
Vitek (BioMerieux)	3
BDPhoenix (Becton Dickinson)	2
Micronaut system (Merlin)	1
16S rDNA sequencing	1
<i>B. multivorans</i>	
Conventional biochemical tests	2
GLC-FAME ^a	1
Api 20NE (BioMerieux)	10
ATB 32 GN (BioMerieux)	1
BBL crystal enteric (Becton Dickinson)	1
Vitek (BioMerieux)	1
BDPhoenix (Becton Dickinson)	2
Micronaut system (Merlin)	1
16S rDNA sequencing	3
recA sequencing	2
<i>P. aeruginosa</i> 1	
Conventional biochemical tests	3
GLC-FAME ^a	1
Api 20NE (BioMerieux)	6
ID 32GN (BioMerieux)	1
BBL crystal enteric (Becton Dickinson)	1
Vitek (BioMerieux)	2
BD Phoenix (Becton Dickinson)	2
Micronaut system (Merlin)	1
16S rDNA sequencing	1
<i>P. aeruginosa</i> 2 ^b	
Api 20 NE (Bio Merieux)	2
BD Phoenix (Becton Dickinson)	1

^a Gas-liquid chromatography analysis of bacterial fatty acid methyl esters.

^b *P. aeruginosa* 2 was correctly identified only by 3 laboratories.

complex by all 17 laboratories and further specified as *B. multivorans* by two laboratories (one of them using *recA* sequencing and the other using 16S rDNA sequencing) but was misidentified as *Burkholderia cenocepacia* by the third laboratory based on *recA* sequencing. *P. aeruginosa* 2 was detected only by four of 17 laboratories (23.5%) and correctly

Table 3
Antimicrobial susceptibility testing methods used by participating laboratories^a

Susceptibility testing methods	No. of laboratories
Agar dilution method	2
Disc diffusion method	11
E-test method	2
Vitek (BioMerieux)	8
BD Phoenix (Becton Dickinson)	2
Micronaut system (Merlin)	3
Interpretation guidelines ^b	
DIN	8
CLSI	8

^a Ten laboratories used two different test methods.

^b One laboratory did not report on interpretation guidelines.

identified as *P. aeruginosa* only by three of them using either API 20NE System (BioMerieux) or the BD Phoenix automated system (Becton Dickinson). The fourth laboratory misidentified *P. aeruginosa* 2 as *Ralstonia picketti* using API 20NE System (Code: 1040535), possibly by reading the API 20NE code only once after 24 h of incubation. Thus, 13 laboratories did not detect *P. aeruginosa* 2. Misidentification of *B. multivorans* as *B. cenocepacia* and overlooking of *P. aeruginosa* 2 with MDR phenotype might have great impact on the clinical treatment and outcome of a CF patient.

3.2. Laboratory cultivation protocols

All 17 laboratories prepared cultivation media by streaking reconstituted freeze-dried samples using loop inoculation, while two laboratories in addition plated serial dilutions of the reconstituted samples and reported on CFU/ml of individual pathogens. Five of 17 laboratories did not use *B. cepacia* complex selective media and nine of 17 laboratories did not use selective media for the detection of *S. aureus*. One laboratory used a meropenem containing

Table 4
Consiliary laboratories and participants antimicrobial susceptibility testing results^a

	MICs/categories designated by		No. of laboratories reporting strains ^b			% of labs correct
	CLNth	CLSth	S	I	R	
	Microdilution	Agar dilution				
<i>S. aureus</i>						
Ciprofloxacin	0.5/S	< 0.5/S	16	0	0	100
Tobramycin	1.0/S	0.5/S	13	0	0	100
Meropenem	< 0.125/S	< 1.0/S	13	0	0	100
Fosfomycin	– ^c /S	16.0/S	6	1	3	60.0
Gentamicin	0.5/S	– ^c /S	16	0	0	100
Cotrimoxazole	< 8.0/S	– ^c /S	16	0	0	100
Erythromycin	> 8.0/R	– ^c /R	2	0	12	85.7
Amoxicillin/clavulanic acid	1.0/S	– ^c /S	14	2	0	87.5
Oxacillin	1.0/S	– ^c /S	17	0	0	100
<i>B. multivorans</i>						
Ciprofloxacin	1.0/S	2.0/I	10	5	2	88.2
Colistin	– ^c /R	> 16.0/R	0	0	14	100
Ceftazidime	2.0/S	2.0/S	17	0	0	100
Tobramycin	32.0/R	> 8.0/R	0	1	16	94.0
Meropenem	2.0/S	2.0/S	11	2	3	68.8
Fosfomycin	– ^d	> 256.0/R	0	0	7	100
Gentamicin	> 32.0/R	– ^c /R	0	0	15	100
Cotrimoxazole	16.0/S	– ^c /S	15	2	0	88.2
<i>P. aeruginosa</i> 1						
Ciprofloxacin	4.0/R	2.0/I	1	4	12	94.1
Colistin	– ^c /S	2.0/S	14	1	0	93.3
Ceftazidime	1.0/S	< 1.0/S	17	0	0	100
Tobramycin	1.0/S	1.0/S	12	4	0	75.0
Meropenem	0.25/S	< 1.0/S	16	0	0	100
Fosfomycin	– ^d	128.0/R	2	0	2	50.0
Gentamicin	4.0/I	– ^c /I	3	10	4	58.8
<i>P. aeruginosa</i> 2 ^c						
Ciprofloxacin	1.0/S	< 0.5/S	3	0	1	75.0
Colistin	– ^c /S	2.0/S	3	0	0	100
Ceftazidime	8.0/I	> 32.0/R	1	2	1	75.0
Tobramycin	4.0/I	4.0/I	0	3	1	75.0
Meropenem	8.0/I	32.0/R	1	1	2	75.0
Fosfomycin	– ^d	128.0/R	0	0	0	
Gentamicin	32.0/R	– ^c /R	0	0	4	100

^a Abbreviations: S, sensitive; I, intermediate; R, resistant; CLNth, German consiliary laboratory for CF bacteriology north; CLSth, German consiliary laboratory for CF bacteriology south.

^b Several substances were not tested by all laboratories.

^c *P. aeruginosa* 2 was detected only by 4 laboratories.

^d Substance was not tested by this laboratory.

^e MIC values were not determined.

agar to detect MDR variants. The reported incubation conditions (duration and temperature) of the primary plates varied distinctly from 48 h to 7 d and 35 °C to 37 °C, respectively. Three laboratories did not report on incubation temperature. Two laboratories reported incubation of *B. cepacia* complex selective media at 22 °C and 30 °C, respectively. Six laboratories used prolonged incubation for *B. cepacia* complex selective media and/or fungal selective media up to 7 days at RT.

All four laboratories successfully cultivating *P. aeruginosa* 2 used loop inoculation for preparation of cultivation media and 48 h incubation, two at 36 °C and one of them at 35 °C, while one laboratory did not report on the incubation temperature. Surprisingly, the two laboratories using serial dilutions, suggesting improved recovery of different species and morphotypes from CF samples, and the single laboratory using Meropenem agar for selection of MDR phenotypes also missed *P. aeruginosa* 2.

3.3. Antimicrobial susceptibility testing

AST methods applied by participating laboratories and reported category results (S/I/R) are shown in Tables 3 and 4. Percentages of correct results were calculated based on AST data of CLSth and CLNth. Differences in category results of CLSth and CLNth occurred only for *B. multivorans* and *P. aeruginosa* 1, both for ciprofloxacin and *P. aeruginosa* 2 for ceftazidime and meropenem, most likely since corresponding MIC values clustered near the AST breakpoints (Table 4). In such cases, both results were accepted as correct. The evaluation of the questionnaires revealed that eight laboratories performed AST according to the standard of the German Institute for Standardization (DIN) and eight laboratories according to American CLSI guidelines. One laboratory did not report on the used AST guidelines.

Although reported only by some laboratories, testing of fosfomycin caused several errors when *S. aureus* and *P. aeruginosa* 1 were tested, not surprisingly, since the disc diffusion method that was used by the majority of laboratories is often inaccurate and to date neither approved CLSI nor DIN breakpoints for fosfomycin are available [23–25].

Furthermore, errors in category results occurred most frequently for meropenem when *B. multivorans* was tested (only 68.8% correct results) and for gentamicin when *P. aeruginosa* 1 was tested (58.8% correct results). Among laboratories reporting categorical errors for meropenem and gentamicin, two laboratories used the Micronaut automated system (Merlin), two laboratories used Vitek automated system (BioMerieux), one laboratory used BD Phoenix automated system (Becton Dickinson), one laboratory used the E-Test (Viva Diagnostics) and three laboratories used the agar diffusion method. One laboratory did not report on its AST method. Three laboratories all of them using the Micronaut system, reported major errors (a susceptible substance was reported resistant) for meropenem when *B. multivorans* was tested, although tested MIC values were

within the susceptible range. The Micronaut system expert software categorically assesses *B. cepacia* complex isolates as meropenem resistant. Thus, evaluation of the questionnaires revealed that discrepancies in AST occurred most frequently when automated systems (Merlin, Vitek, BD Phoenix) or the agar diffusion method were used.

4. Discussion

Accurate identification and AST of CF pathogens is critical to ensure appropriate treatment and infection control in CF patients. The present study provides a first status on microbiological practices for CF specimens in German microbiological laboratories. All laboratories participating in this EQA trial are experienced in CF microbiology and associated to centres following at least 100 CF patients. However, only three from 17 laboratories (18%) reported fully correct results concerning the cultivation and identification of a set of four typical CF pathogens from simulated clinical specimens. All laboratories isolated and identified three of the four test strains, namely *S. aureus*, *P. aeruginosa* 1 and *B. cepacia* complex correctly, while *P. aeruginosa* 2 with an MDR phenotype was problematic for the majority of participants. Only four laboratories correctly reported the presence of this strain, but one misidentified it as *R. pickettii*. Three laboratories determined the genomic species of the *B. cepacia* complex test strain, two of them correctly as *B. multivorans*, but one laboratory misidentified it as *B. cenocepacia*. This reporting error may have fatal implications on the patient's clinical therapy strategies and prognosis, as *B. cenocepacia* infection is associated with a worse outcome, especially following lung transplantation, than those with *B. multivorans* [26]. Likewise, overlooking of *P. aeruginosa* 2 could result in the selection of an ineffective antibiotic treatment.

The evaluation of the questionnaires revealed a broad heterogeneity between microbiological protocols regarding cultivation and AST procedures. Cultivation procedures implemented for non-CF specimens may fail to detect fastidious species found in CF specimens like *B. cepacia* complex, slow growing mucoid *P. aeruginosa* or small colony variants (SCV) that can easily be overgrown by other micro-organisms. Failure in the recovery of these CF pathogens may negatively influence clinical treatment, as probably important etiologic pathogens or more resistant components of the infection will be missed. Further, the omission or the delayed separation of infected patients may lead to an increased risk of patient-to-patient transmission.

For the primary processing of CF respiratory specimens the use of selective media and prolonged incubation periods are essential. For the optimal recovery of gram-negative bacteria, mainly *P. aeruginosa*, selective media such as Pseudomonas isolation, cetrimide, MacConkey or acetamide agar are required. To improve the recovery of *H. influenzae*, chocolate agar supplemented with vancomycin and/or bacitracin and clindamycin is recommended [15]. Due to

the high transmissibility of some *B. cepacia* complex species and their association with a poor prognosis, the use of isolation media with enhanced recovery rate for this organism are strictly recommended (e.g., MAST selective agar, *B. cepacia* selective agar, *Pseudomonas cepacia* agar, oxidation-fermentation polymyxin bacitracin lactose agar, Stewart's composite arginine medium) [16–19]. To ensure the reliable recovery of *S. aureus*, mannitol salt agar or other media have been used (e.g., Columbia colistin-nalidixic acid agar, lipase-salt-mannitol agar). For the recovery of *S. aureus*, SCVs chromogenic agar media were shown to be reliable (e.g., *S. aureus* ID agar, CHROMagar Staph aureus) [27]. Detection of MDR variants is facilitated by agar media supplemented with antibiotics, like meropenem, tobramycin or colistin [28–31].

As a consequence, in CF selective media have been advocated by American authorities for the optimum recovery of *H. influenzae*, *S. aureus*, *P. aeruginosa* and *B. cepacia* complex [19]. Surprisingly, only eight of 17 laboratories used selective media for *S. aureus* and 12 of 17 laboratories for *B. cepacia* complex. Although the high bacterial load of *S. aureus* and *B. multivorans* most likely enabled all laboratories to detect both strains without usage of specific selective media, these pathogens could be missed when lower CFU are present and/or more rapidly growing species (e.g., *P. aeruginosa*) obscure their detection [32]. Likewise, the overlooking of *P. aeruginosa* 2 appear to be the result of the more prominent colony size and/or slightly higher cell count of *P. aeruginosa* 1, *S. aureus* and *B. multivorans*. While almost all participating laboratories prepared bacterial cultures only by loop-inoculation of undiluted samples, quantitative cultures performed by CLSth and CLNth demonstrated that *P. aeruginosa* 2 was present in easily detectable amounts. This underlines the need of adequate sample homogenization and whole plate inoculation of serial dilutions to efficiently separate different CF pathogens and phenotypic variants from the more challenging viscous CF airway secretions that in contrast to the homogeneous freeze-dried sample contain heterogeneously distributed bacteria in biofilm-like microcolonies.

For species identification, the majority of laboratories used biochemical methods while three laboratories confirmed *B. cepacia* complex identification by 16S rDNA sequencing. Several studies have shown that conventional biochemical tests can misidentify *B. cepacia* complex species, biochemical inactive and newly emerging CF pathogens [6,33]. Thus, laboratories processing CF specimens need access to molecular identification tools and/or in equivocal cases the possibility to send specimens and/or isolates to national or international referral laboratories on CF microbiology [34]. Services regarding the genomic species identification of *B. cepacia* complex isolates are available by the European *B. cepacia* complex Referral Laboratory and Repository (information via <http://go.to/cepacia>).

Moreover, accurate AST in particular of highly resistant CF isolates plays a critical role in the diagnostic microbi-

ology [35]. The evaluation of the questionnaires revealed that most laboratories used either automated commercial systems (13 of 17 laboratories) and/or the disc diffusion method (11 of 17 laboratories), whereas either CLSI or DIN guidelines were used. Most categorical errors occurred when automated systems were applied followed by the agar diffusion test. The overall strain-specific error rates for AST results were found to be as follows: *P. aeruginosa* 2 *B. multivorans* *P. aeruginosa* 1 > *S. aureus* (excluding fosfomycin due to the absence of clear AST guidelines for this agent). Categorical errors occurred mostly when MIC values of the test strains clustered near the corresponding breakpoints. Very major errors (a resistant substance was reported susceptible) that may lead to an ineffective antibiotic treatment occurred only for *P. aeruginosa* 1 when fosfomycin was tested and surprisingly, twice for *S. aureus* when erythromycin was tested. The highest number of major errors occurred for *B. multivorans* when meropenem was tested, probably due to an algorithm of the Micronaut expert software (Merlin) that generally scores *B. cepacia* isolates as meropenem resistant.

In general, instead of the labor-intensive agar dilution method mainly the broth microdilution method and the agar-bond disk diffusion test have been considered to be appropriate for the AST of CF strains [36,37]. Nevertheless, the disc diffusion testing of fastidious CF isolates, especially for mucoid *P. aeruginosa*, may be inaccurate [36]. The use of automated commercial systems (Vitek and MicroScan-WalkAway) for testing CF pathogens was shown to be inaccurate and thus has not been recommended by American authorities [38]. The wide use of automated systems among participants may account for a high number of reported AST errors. However, up-to-date studies evaluating newer generations of automated systems in the field of CF microbiology are not available. The Merlin Micronaut system was shown to be reliable for *P. aeruginosa* CF isolates [39,40]. In 2005 revised CLSI guidelines have been released that for the first time include an approval of the disc diffusion for the testing of *B. cepacia* complex and *S. maltophilia* when prolonged incubation times and adapted breakpoints are used. Nevertheless, the most suitable AST method of isolates obtained from CF patients is increasingly under debate. Beyond methodological discrepancies the poor correlation of *in vitro* data and the outcome of the patients is disappointing, irrespective of the testing of single antibiotics or antibiotic combinations [41–43]. This seems to be not surprisingly, since (i) the definition of resistance deduced from *in vitro* testing of bacteria and discrete clinical breakpoints is directed in particular towards the treatment of acute infections, (ii) in CF only a few possibly representative isolates were tested and (iii) rather all *in vitro* methods are inappropriate to mimic the complex environment of the CF lung comprising various sputum components, high bacterial densities, biofilm growth, oxygen restriction and the coexistence of different species or phenotypic variants [44].

Nevertheless, until adequate AST protocols that provide a more rational basis for prescribing antibiotics for CF lung infection are missing, reliable conventional AST data should not be rejected since they may be still essential to guide antibiotic therapies during early CF disease and exacerbations and to unmask multiresistant isolates with respect to epidemiological and hygienic purposes [45,46].

In conclusion, this study highlighted that national EQA schemes are important to assess and improve the microbiological performance of CF respiratory tract specimens. As a part of this project, recommendations for German clinical microbiology laboratories processing CF respiratory secretions were recently published [21]. Probably yearly conducted EQA schemes are desirable to periodically evaluate the compliance of microbiological laboratories to guidelines that should be regularly updated, e.g., with respect to changes in the epidemiology of CF lung infection.

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